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Biosorption of Reactive Black 5 dye by *Penicillium restrictum*: The kinetic study

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Abstract

Biosorption of Reactive Black 5 (RB 5) dye onto dried *Penicillium restrictum* biomass was studied with respect to pH, contact time, biosorbent and dye concentrations. The effect of temperature on the biosorption efficiency was also carried out and the kinetic parameters were determined. Optimum initial pH, equilibrium time and biomass concentration for RB 5 dye were found to be 1.0, 75 min and 0.4 g dm^{-3} at 20 °C, respectively. The maximum biosorption capacities (q_{max}) of RB 5 dye onto dried *P. restrictum* biomass were 98.33 and 112.50 mg (g biomass)$^{-1}$ at 175 mg dm^{-3} initial dye concentration at 20 and 50 °C, respectively, and it was 142.04 mg (g biomass)$^{-1}$ at 200 mg dm^{-3} initial dye concentration at 35 °C. The results indicate that the biosorption process obeys a pseudo-second-order kinetic model.

Keywords: *Penicillium restrictum*; Biosorption; Reactive Black 5

1. Introduction

Dyes are synthetic chemical compounds having complex aromatic structures. They contain different chromophores such as azo groups, which combine with various reactive groups [1]. They are classified as acidic, basic, azo, diazo, disperse, metal complex and antraquinone-based dyes [2] according to their structural varieties and generally considered as a primary contributor for the environmental pollution due to their wide use in many areas especially in textile industry. The major industries utilizing dye molecules to colour their final products in addition to textiles are dye houses, cosmetics, food, rubber, leather, pharmaceutical, paper and printing industries [3]. The hazardous effects of dyes come from their discharge into receiving waters. Once they are released, they not only produce toxic amines by the reductive cleavage of azo linkages which causes severe effects on human beings through damaging the vital organs such as the brain, liver, kidneys, central nervous and reproductive systems [4,5] but also prevent photosynthetic activity in aquatic life by reducing light penetration [6]. Therefore, their removal causes a big environmental concern in industrialized countries in the world and is subjected to many scientific researches.

Commonly used traditional methods to eliminate dyestuffs from textile and dye-containing effluents are those of activated carbon adsorption, reverse osmosis, oxidation, ultra filtration, flocculation, color irradiation, coagulation, sedimentation and precipitation [7], but they are ineffective, especially for the removal of brightly coloured, water-soluble reactive and acid dyes [8]. This is because dyes show resistance to many chemicals, oxidizing agents and light [9]. The activated carbon adsorption is of choice because of its high adsorption capacity and surface area as well as having microporous structures [10], but its large scale application is restricted due to high operating costs, problem with regeneration and relatively high price [6,11].

Biosorption process is attracted great attention in recent years as less costly alternative methods in place of current adsorption processes since they utilize not only plant materials [10] but also a wide variety of microorganisms in dead, pretreated and immobilized forms as adsorbing agents [12]. These materials are cheap to produce and carry wide range of binding sites for dye molecules [13]. Therefore they are subjected to many researches to be investigated for the removal of various dyes from aqueous solutions such as Acid Red 274 [14], Basic Blue 41 [11], Rhodamine B [15], Congo Red [16], Methylene Blue

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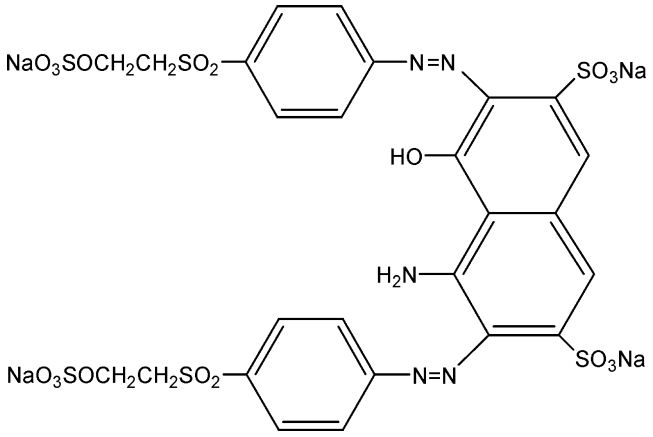

Fig. 1. The chemical structure of Reactive Black 5.

[17] and Acid Red 57 [18]. The term "biosorption" refers to the removal of unwanted organic and inorganic species, which include dyes, metals and odor causing substances by microbial biomass through a combination of active and passive transport mechanisms including ion-exchange and complexation [19,20]. Microbial cell surfaces carry various types of functional groups of amino, carboxylate, phosphate and hydroxyl which are responsible for the sequestration of hazardous materials from industrial effluents [21].

In this study, biosorption of RB 5 dye (see Fig. 1), commonly used in textile industry for coloring clothes in Turkey, onto dried *Penicillium restrictum* biomass was investigated in a batch system with variation in the parameters of initial pH, contact time, dye and biosorbent concentration in addition to temperature. The biosorption kinetic was also investigated. To our knowledge, this is the first example for *P. restrictum* biomass to be used as a biosorbent material for the removal of dye molecules from aqueous solutions.

2. Material and methods

2.1. Preparation of the biosorbent

The filamentous fungus, *P. restrictum* (wild type), was isolated from Industrial Wastewater Treatment Plant in Eskişehir, Turkey. The fungus was stored on potato dextrose agar slants at 4 °C [22]. A medium for growing *P. restrictum* was prepared by mixing sucrose (20 g), bacto peptone (5 g), neopeptone (5 g), KH_2PO_4 (1 g), $NaNO_3$ (1 g) and $MgSO_4{\cdot}7H_2O$ (0.5 g) in distilled water (1 dm^3). The pH of the growth medium was adjusted to 5.5 by the addition of 1 M HCl before autoclaving at 121 °C for at least 20 min. Erlenmayer flasks containing the above media (0.1 dm^3) were inoculated with spore suspension (0.001 dm^3) obtained shaking sterile water (0.01 dm^3) with mature slopes of *P. restrictum* under sterile conditions. Growth was allowed to proceed for 7 days at 25 °C on a rotary shaker operating at 120 rpm. After the fungal growth, the biomass and the culture medium were separated by filteration. The resulting biomass was washed several times thoroughly with distilled water, spread on Petri dishes and dried in an oven at 60 °C overnight. They were then powdered using a mortar and pestle and sieved to select particles 150 μm for use as a biosorbent.

2.2. Preparation of dye solution

The dye used in this study was Reactive Black 5 (RB 5; commercial name Sakazol Black B) obtained from BIRBOY textile company in Istanbul, Turkey and used without further purification. The tests solutions containing RB 5 dye were prepared by diluting 1.0 g dm^{-3} of stock solution which was prepared by dissolving an accurate quantity of dye in distilled water.

2.3. Dye biosorption experiments

Laboratory biosorption experiments were performed at different biomass feeds, initial RB 5 dye concentrations and various temperatures. The batch experiments were carried out in a beaker (0.1 dm^3) at an agitation speed of 200 rpm on a magnetic stirrer. The biosorption capacity was determined by using the following equation taking into the concentration difference of the solution at the beginning and equilibrium accounts:

$$q_e = \frac{[(C_i - C_e)]xV}{m} \quad (1)$$

where C_i and C_e are the initial and the equilibrium dye concentrations (mg dm^{-3}), V is the volume of solution (dm^3) and m is the amount of biosorbent used (g).

Firstly, the effect of the solution pH on the biosorption capacity of RB 5 dye onto dried *P. restrictum* biomass was examined by equilibrating the adsorption mixture with dried biomass (0.02 g) and 0.05 dm^3 of 150 mg dm^{-3} RB 5 dye solution, adjusting the pH value between 1 and 10 adding freshly prepared 0.1 M HCl or 0.1 M NaOH solutions for 1 h. This was followed by the assessment of the effect of equilibrium time varied between 10 and 120 min on the dye biosorption capacity of the biosorbent. Then, the binding capacity of biomass was assessed, varying the RB 5 dye concentration within the range of 100–250 mg dm^{-3} and adjusting the pH to a value of 1.0 which is the optimum pH. The effect of biomass concentration on RB 5 sorption was also determined using biomass samples ranging from 0.02 to 0.2 g at 0.05 dm^3 of 150 mg dm^{-3} RB 5 dye solution and pH of 1.0 for 1 h. When the sorption procedure completed, the solutions were centrifuged at 4500 rpm for 10 min and the supernatants were then analyzed for residual RB 5 dye concentrations spectrophotometrically using a spectrophotometer (UV/vis, Cecil 4002) at λ_{max} 596.0 nm. The solutions concerned were diluted to known concentrations to read the values before making the measurements. The optimum pH and biomass concentration were determined as 1.0 and 0.4 g dm^{-3}, respectively, and used throughout all biosorption experiments.

Finally, several experiments were conducted to study the effects of temperature on the biosorption process and deduce kinetic parameters as follows: a constant biomass of 0.02 g was weighed and mixed with 0.05 dm^3 of 150 mg dm^{-3} RB 5 dye solutions at various time intervals between 10 and 120 min and temperatures of 20, 35 and 50 °C. The concentration of RB 5 dye was determined as described above.

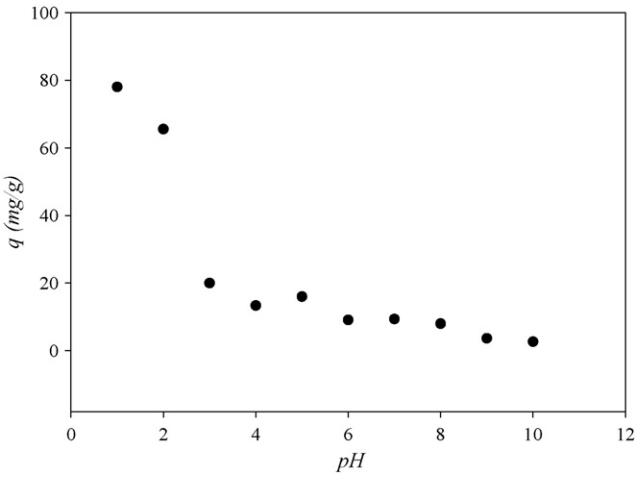

Fig. 2. Effect of pH for the biosorption of RB 5 dyes onto dried *P. restrictum* biomass at 20 °C.

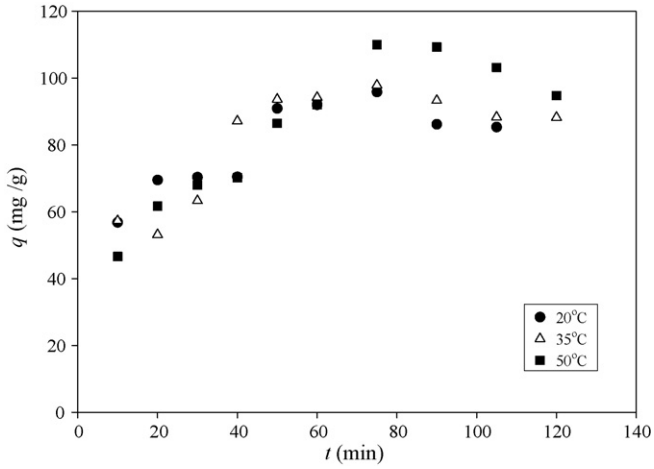

Fig. 3. The effect of equilibrium time for biosorption of RB 5 dyes onto dried *P. restrictum* biomass at temperatures of 20, 35 and 50 °C.

3. Results and discussion

3.1. Effect of pH

The pH is an important parameter for biosorption studies and affects not only the biosorption capacity, but also the colour and solubility of dye solutions. The maximum biosorption capacities of dried *P. restrictum* biomass are plotted against solution pH in Fig. 2 using 0.05 dm^3 of 150 mg dm^{-3} initial dye solution, 0.02 g biomass concentration, contact time of 60 min at 20 °C. As shown in this figure, the equilibrium biosorption capacity of the biosorbent decreased from 78.00 to 65.50 mg (g biomass)$^{-1}$ when the solution pH is changed from 1 to 2 and a sharp decrease was observed at pH 3, dropping the biosorption capacity to 20.00 mg (g biomass)$^{-1}$. This trend is continued with increasing solution pH from 4 to 8, causing the equilibrium uptake capacity to drop from 13.35 to 8.00 mg (g biomass)$^{-1}$. The sorption capacity further decreased after pH 8 and reaches the lowest level of 2.68 mg (g biomass)$^{-1}$ biosorption capacity at pH 10. From this study, the optimum pH is determined as 1 at which the maximum biosorption capacity of dried *P. restrictum* biomass for RB 5 dyes was determined as 78.00 mg (g biomass)$^{-1}$ at 20 °C. This effect is largely related to the anionic characters of RB 5 dye. Weak base groups in the biomass surface are protonated and acquire a net positive charge with diminishing solution pH. This causes a significantly high electrostatic attraction between the surface of dried *P. restrictum* biomass and RB 5 dyes, resulting in a high biosorption capacity. Lower biosorption capacity of RB 5 observed at basic pH is a result of competition between the excess hydroxyl ions and the negatively charged dye ions for the biosorption sites [13].

In a study describing the removal of RB 5 dye from aqueous solutions by dried activated sludge, Gulnaz et al. [23] reported that the optimum solution pH was 2 at which the adsorption capacity of the dried sludge was determined as 116 mg g^{-1} for 20 °C.

3.2. Effect of contact time

Contact time is one of the important parameters for successful deployment of the biosorbents for practical application and rapid sorption is among desirable parameters [24]. Fig. 3 indicates the RB 5 dye uptake by the biosorbent as a function of contact time at different temperatures of 20, 35 and 50 °C. An uptake capacity of 56.83 mg (g biomass)$^{-1}$ was observed within 10 min and then the sorption capacity was increased constantly with increasing contact time reaching to a maximum point of 95.83 mg (g biomass)$^{-1}$ in 75 min at 20 °C. Beyond the equilibrium time, there is a steady decrease observed on the biosorption capacity. A similar trend was observed at 35 and 50 °C and the maximum biosorption capacities were determined as 97.92 and 110.00 mg (g biomass)$^{-1}$ in 75 min, respectively, followed by steady decrease with increasing the contact time. Therefore 75 min is fixed as the optimum contact time for studies carried out at 20, 35 and 50 °C. An increase observed on the biosorption capacity with increasing contact time is due to availability of biosorption sites on the biomass surface. A decrease observed on the biomass capacity after equilibrium time could be related to the desorption of dye molecules from the biomass surfaces probably caused by repulsive forces between dye molecules at adjacent sites on the biomass surfaces [25].

3.3. Effect of biosorbent concentration on RB 5 dye removal

The biosorption of RB 5 dye onto dried *P. restrictum* biomass was measured at seven different biosorbent concentration at pH of 1 and contact time of 75 min and 20 °C, using 0.05 dm^3 of 150 mg dm^{-3} dye solution to investigate the effect of biosorbent concentrations. The results of the experiments are presented in Fig. 4. It is clear from the figure that the biosorbed dye concentration was decreased from 85.92 to 35.98 mg (g biomass)$^{-1}$ with increase in biosorbent concentration, from 0.4 to 4.0 g dm^{-3}. The decrease in biosorption capacity with increasing biosorbent concentration could be explained by not

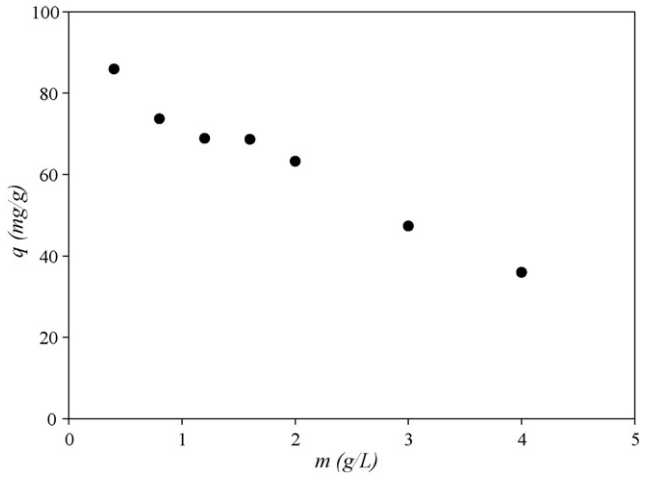

Fig. 4. Effect of biosorbent concentration for biosorption of RB 5 dyes onto dried *P. restrictum* biomass at 20 °C.

only unsaturation of biosorption sites through the adsorption reaction but also the particle interaction such as aggregation occurring at high biosorbent concentration and leading to decrease in total surface area [26]. Another reason could be due to the splitting effect of concentration gradient between dye molecules and biomass concentration causing a decrease in the amount of dye biosorbed onto unit weight of biomass [27].

3.4. Effect of initial dye concentration

The parameter, initial concentration, provides an important driving force to overcome resistances encountered when all molecules are transferred between the aqueous and solid phases [28]. In this study, the RB 5 dye removal capacity of dried *P. restrictum* biomass was investigated using RB 5 dye solutions ranged from 100 to 250 mg dm^{-3} at pH 1.0 and 20 °C. The equilibrium dye uptake capacity value (mg (g biomass)$^{-1}$) is given in Fig. 5. The equilibrium loading capacity increased from 73.92 to 100.46 mg (g biomass)$^{-1}$ as the initial dye concentration was increased from 100 to 175 mg dm^{-3} which is the maximum dye uptake value at 20 °C. Then the biosorption capacity decreased to a value of 81.04 mg (g biomass)$^{-1}$ as the initial dye concentration was further increased to 250 mg dm^{-3}. Therefore 175 mg dm^{-3} dye concentration is determined as the optimum initial dye concentration at 20 °C. The effects could be explained as follows: at lower initial dye concentrations, all dye molecules could interact with the binding sites on the biomass surface and high sorption rates occur while at high initial dye concentrations, binding sites on the biomass surface are saturated and no further biosorption occurs. A decrease observed on the biosorption capacity is mainly due to the repulsive forces between dye molecules at adjacent sites on the cell surface, resulting in removal of some dye molecules from the surface [25].

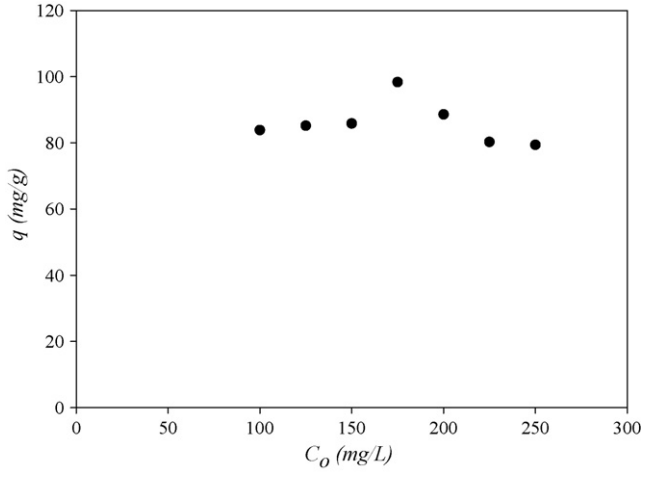

Fig. 5. The effect of initial RB 5 dye concentration for biosorption of RB 5 dyes onto dried *P. restrictum* biomass at 20 °C.

3.5. Effect of temperature

The temperature has two main effects on the sorption processes. Increasing temperature is known to increase the diffusion rate of the adsorbate molecules within the pores as a result of decreasing solution viscosity and will also modify the equilibrium capacity of the adsorbent for a particular adsorbate [29]. To investigate the effect of temperature, the equilibrium biosorption capacity of RB 5 dye onto dried biomass of *P. restrictum* was studied at three constant temperatures of 20, 35 and 50 °C. An increase in the temperature from 20 to 35 and to 50 °C led to an increase on the uptake capacity of the biomass for the dye molecules from 100.46 to 110.88 and 112.50 mg (g biomass)$^{-1}$, respectively, under optimum condition of pH, biomass concentration and equilibrium time at an initial concentration of 175 mg dm^{-3}, respectively. This result indicated that a better biosorption of RB 5 dye is actually obtained at higher temperatures after the equilibrium time.

3.6. Biosorption kinetic

The kinetics studies have carried out to determine the efficiency of RB 5 dye biosorption onto dried *P. restrictum* biomass and indicated that the biosorption capacity increases with the initial dye concentrations in all cases. Various kinetic models including first-order and pseudo-second-order were tested for the experimental data to elucidate the biosorption mechanism.

The pseudo-second-order kinetic model [30] is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_2^2} + \frac{1}{q_2} t \tag{2}$$

where q_2 is the biosorbed dye amount at equilibrium (mg g^{-1}) for the pseudo-second-order biosorption, q_t is the amount of RB 5 dye biosorbed at time t (mg g^{-1}) and k_2 is the pseudo-second-order kinetic rate constant (g mg^{-1} min^{-1}). Values of k_2 and q_2 were calculated from the plot of t/q_t against t (Fig. 6).

The plots of linear form of the pseudo-first-order and pseudo-second-order were obtained at the temperatures of 20, 35 and 50 °C and the kinetic parameters for the biosorption process are given in Table 1. The plots of $1/q_t$ versus $1/t$ for the first-order equation are not shown as a figure because the correlation coefficients for the pseudo-first-order model are lower than that of

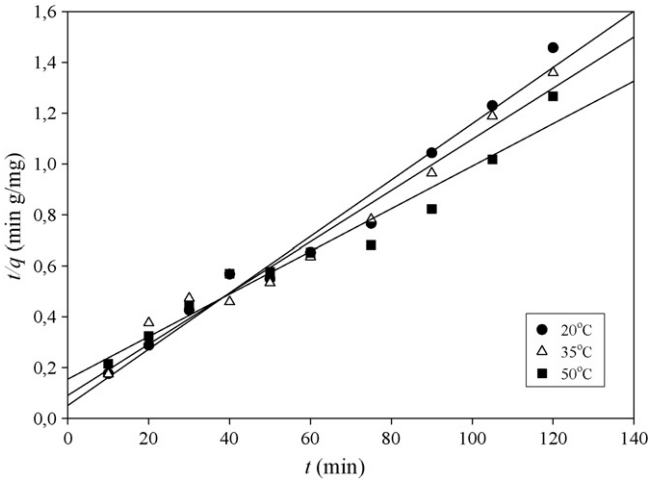

Fig. 6. Pseudo-second-order kinetic plots for biosorption of RB 5 dye onto dried *P. restrictum* biomass at temperatures of 20, 35 and 50 °C.

Table 1
Pseudo-second-order kinetic parameters for the biosorption of RB 5 dyes onto dried *P. restrictum* biomass at temperatures of 20, 35 and 50 °C

t (°C)	k_2 (g mg^{-1} min^{-1})	q_2 (mg g^{-1})	q_{exp} (mg g^{-1})	r_2^2
20	2.392×10^{-3}	90.25	95.83	0.978
35	1.111×10^{-3}	99.40	97.92	0.974
50	4.538×10^{-4}	119.43	110.00	0.958

the pseudo-second-order model. The magnitude of the regression coeffcient r^2 for the pseudo-second-order model changed between 0.978 and 0.958 at constant temperatures of 20, 35 and 50 °C. Therefore, this implies that the biosorption of RB 5 dyes onto dried *P. restrictum* biomass does not follow first-order kinetic, but follow the pseudo-second-order kinetic models.

4. Conclusions

In the present study, the biosorption behavior of a reactive dye, namely Reactive Black 5 (RB 5) onto dried *P. restrictum* biomass has been investigated with variations in parameters of pH, biosorbent and dye concentrations and contact time at various temperatures. The maximum biosorption capacity for RB 5 dye was found to be 142.04 mg (g biomass)$^{-1}$ under optimum conditions of pH 1, contact time of 75 min, biomass concentration of 0.02 g at initial concentration of 200 mg dm^{-3} and 35 °C. The biosorption process was found to obey a pseudo-second-order rate. Increasing temperature caused an increase on the biosorption capacity of the dried cell, indicating that higher temperatures favor the biosorption process. In the light of these experimental results, it can be concluded that the filamentous fungus, *P. restrictum* in dried form has a potential to be used as an alternative biosorbent material for the removal of RB 5 dye from aqueous solutions because of easily cultivable, its low cost, reasonable biosorption capacity with low biomass dosage (0.4 g dm^{-3}) and being free from pathogenicity.

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